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RESOLUTION OF STEREOISOMERS OF ω2-HYDROXY ACIDS AND 2-ALKANOLS BY GAS-LIQUID CHROMATOGRAPHY

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A number of compounds possessing the $-CH(OH)CH_3$ group have been reacted with (R)-1-phenylethyl isocyanate. Each hydroxy compound yielded a pair of diastereoisomeric *N*-(1-phenylethyl) urethanes, which were separated by gas-liquid chromatography using 1% QF-1 as stationary phase.

Introduction

The formation of ω2-hydroxy acids*, i.e. fatty acids carrying a hydroxyl group at the penultimate carbon atom, has been reported for a number of tissues and species. Thus octadecanoic acid is converted into 17(*S*)-hydroxyoctadecanoic acid by yeast of the genus *Torulopsis*[1]). The conversion of 5,8,11,14-eicosatetraenoic acid into a mixture of 19-hydroxy- and 18-hydroxy-5,8,11,14-eicosatetraenoic acids by *Ophiobulus graminis* was recently reported[2]). Furthermore, ω2-hydroxy acids are formed together with ω1-hydroxy acids by ω-hydroxylation of fatty acids in the rat, both in vivo[3, 4]) and in vitro[5]). Analogous compounds, viz. prostaglandins hydroxylated at the penultimate carbon atom, have been isolated from human seminal plasma[6, 7]) and have also been obtained by ω-hydroxylation using a preparation of guinea pig liver[8]). A number of 2-alkanols, e.g. 2(*S*)-octadecanol and 2(*S*)-eicosanol have been isolated from certain *Mycobacteria*[9]).

The assignment of the absolute configuration of such hydroxy compounds presents a problem in those cases where the amounts available are too small to permit measurement of the optical rotation. The present paper describes the gas-liquid chromatographic separation of diastereoisomeric urethanes formed by reaction of compounds containing the $-CH(OH)CH_3$ group with (*R*)-1-phenylethyl isocyanate. This reagent has earlier been used for the

* The carbon atom of the terminal methyl group of the fatty acid or ester is designated "ω1".

separation of urethanes of a number of secondary alcohols by thin layer chromatography[10]).

Materials

17-Hydroxyoctadecanoic acid was obtained as described by Bergström et al.[11]). Methyl 17(*R*)-hydroxyoctadecanoate and methyl 17(*S*)-hydroxyoctadecanoate were generous gifts of Dr. A. P. Tulloch, Prairie Regional Laboratory, Saskatoon, Saskatchewan, Canada (cf.[12]). 2(*R*)- and 2(*S*)-eicosanols and 2(*R*)- and 2(*S*)-tetracosanols were kindly given by Dr. K. Serck-Hanssen, Institute of Medical Biochemistry, University of Gothenburg, Göteborg, Sweden (cf.[13]).

1. Methyl 19-hydroxyeicosanate

A mixture of 17-acetoxyoctadecanoic acid (0.3 mmoles) and monomethyl succinate (3 mmoles) in 25 ml of methanol containing 0.1 mmole of sodium methoxide was electrolysed for 1 hour (current, 0.4 A). The reaction mixture was saponified and was then re-esterified by treatment with diazomethane. Silicic acid chromatographic separation of this material (column, 10 g; eluent, 300 ml of 15% ether in hexane) yielded crude methyl 19-hydroxyeicosanate. Subsequent purification by preparative thin layer chromatography (Silica Gel G; solvent system, 50% ether-petroleum ether) afforded 30 μmoles of pure methyl 19-hydroxyeicosanate ($R_f = 0.58$; reference, methyl 17-hydroxyoctadecanoate, $R_f = 0.55$). The identity of the compound with methyl 19-hydroxyeicosanate was based on 1) the method of its preparation by a well established procedure for two-carbon elongation; 2) behaviour on gas-liquid chromatography using 1.4% SE-30 and 1% QF-1 (C-values†, found C-22.0 (SE-30) and C-24.0 (QF-1); reference, methyl 17-hydroxyoctadecanoate, C-20.0 (SE-30) and C-22.0 (QF-1)) (cf. also[14])); and 3) mass spectrometric analysis. The mass spectrum showed ions of high intensity at *m/e* 324 (M-18; loss of water), 298 ($CH_3{-}O{-}CO{-}(CH_2)_{17}\cdot$ plus H·), 295, 292 (M-(18+32); loss of water and methanol), 255, 250, 199, 185, 171, 143, 87, 74, and 45 ($\cdot CH(OH)CH_3$). This fragmentation pattern is in complete accord with that expected for a C_{20} methyl ester hydroxylated at C-19 (cf.[15]).

2. Methyl 21-hydroxydocosanate

Anodic coupling of 17-acetoxyoctadecanoic acid (0.3 mmoles) and monomethyl adipate (3 mmoles), saponification and re-esterification, and subsequent silicic acid chromatographic separation as described above, yielded

† The retention times were related to the retention times of standards of methyl esters of straight chain fatty acids (cf. 14).

crude methyl 21-hydroxydocosanate. The pure hydroxyester (25 μmoles) was obtained after preparative thin layer chromatography ($R_f = 0.61$; reference, methyl 17-hydroxyoctadecanoate, $R_f = 0.55$). The retention times obtained on gas-liquid chromatography corresponded to C-24.0 (SE-30) and C-26.0 (QF-1), and the mass spectrum showed ions of high intensity at *m/e* 352 (M-18; loss of water), 326 ($CH_3-O-CO-(CH_2)_{19}\cdot$ plus $H\cdot$), 323, 320 (M-(18+32); loss of water and methanol), 283, 278, 199, 185, 171, 143, 87, 74, and 45($\cdot CH(OH)CH_3$) (cf.[14]) and [15]).

3. (R)-1-PHENYLETHYL ISOCYANATE

This compound was prepared by treatment of (*R*)-α-phenylethylamine[16]) with phosgene by modification of a procedure earlier used for the synthesis of isocyanates from amines[17]). (*R*)-α-Phenylethylamine, 17 mmoles, was dissolved in 13 ml of dry toluene and slowly added to a solution of 71 mmoles of phosgene in 62 ml of toluene. The mixture was kept at 80° for 16 hours and then at reflux temperature for 2 hours. After cooling, excess of phosgene was removed by bubbling dry nitrogen gas into the solution for 24 hours. The solution of (*R*)-1-phenylethyl isocyanate thus obtained, containing about 0.3 mmole/ml, was stored at +4° over calcium carbonate.

Methods and results

1. PREPARATION OF N-(1-PHENYLETHYL)URETHANES

(*R*)-1-Phenylethyl isocyanate, 30 μmoles (0.1 ml of the reagent solution), was added to the hydroxy compound (3–7 μmoles) and the mixture was kept at 120° for 3 hours under argon. The solvent was then removed using a vacuum pump and the residue was dissolved in ethyl acetate and directly analysed by gas-liquid chromatography. The amount of the reagent was reduced when analysing smaller amounts of hydroxy compounds. Thus 1.5 μmole of (*R*)-1-phenylethyl isocyanate (5 μl of the reagent solution) plus 95 μl of purified xylene were added to the hydroxy compound (0.03 μmoles) and the mixture was treated as described above.

2. GAS-LIQUID CHROMATOGRAPHIC ANALYSIS

The diastereoisomeric urethanes formed by treatment of ω2-hydroxy methyl esters and 2-alkanols with (*R*)-phenylethyl isocyanate were separated using a 1.8 m column packed with 1% QF-1 on Gaschrom Q. Helium was used as carrier gas. A gaschromatogram showing the separation of the derivatives of a mixture of methyl 17-hydroxyoctadecanoate, methyl 19-hydroxyeicosanate and methyl 21-hydroxydocosanate is given in fig. 1. The retention times are given in table 1. The derivatives of methyl 17(*R*)-hydro-

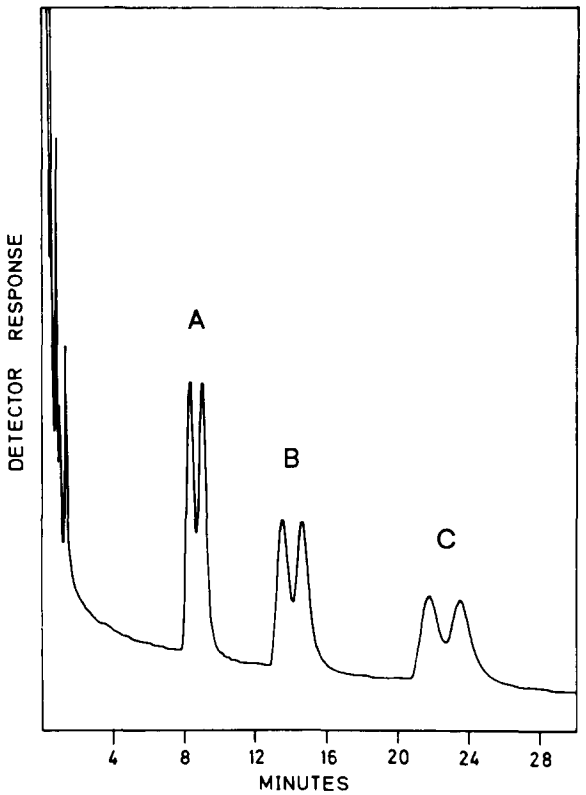

Fig. 1. Separation of the *N*-(1-phenylethyl)urethanes of methyl 17-hydroxyoctadecanoate (A), methyl 19-hydroxyeicosanate (B), and methyl 21-hydroxydocosanate (C).

TABLE 1

Compound	Separation temp., °C	Retention time, min.	Ratio of retention times of diastereoisomeric derivatives
Methyl 17-hydroxyoctadecanoate	210	8.4 (*S*), 9.1 (*R*)	1.08
Methyl 19-hydroxyeicosanate	210	13.6, 14.7	1.08
Methyl 21-hydroxydocosanate	210	21.8, 23.6	1.08
2-Dodecanol	160	4.4, 4.9	1.11
2-Tetradecanol	160	8.3, 9.2	1.11
2-Hexadecanol	160	15.6, 17.5	1.12
2-Eicosanol	200	6.2 (*S*), 6.7 (*R*)	1.08
2-Tetracosanol	200	16.9 (*S*), 18.5 (*R*)	1.09

xyoctadecanoate and methyl 17(*S*)-hydroxyoctadecanoate were also run separately. It was found that the derivative of the 17(*S*)-hydroxy compound had a shorter retention time than the derivative of the 17(*R*)-hydroxy compound (table 1).

The structures of the derivatives were confirmed by mass spectrometric analysis using the LKB 9000 instrument. In the mass spectra of the derivatives of methyl 17-hydroxyoctadecanoate ions of high intensity were present at *m/e* 461 (M), 446 (M-15; loss of $\cdot CH_3$), 402, 297 (M-164; loss of $C_6H_5-CH(CH_3)-NH-CO-O\cdot$), 270($CH_3-O-CO-(CH_2)_{15}\cdot$ plus $H\cdot$), 265 (M-(164+32); loss of $C_6H_5-CH(CH_3)-NH-CO-O\cdot$ plus methanol) 264 (M-(165+32); loss of $C_6H_5-CH(CH_3)-NH-CO-OH$ plus metha-

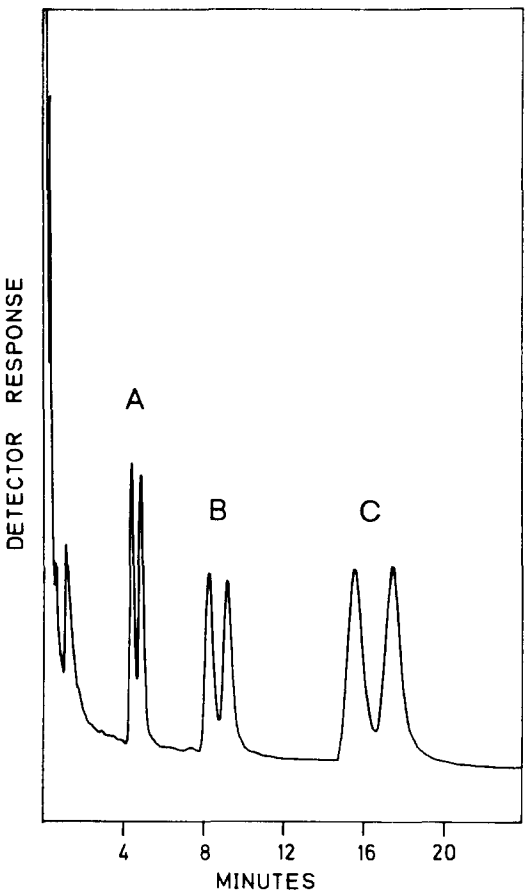

Fig. 2. Separation of the *N*-(1-phenylethyl)urethanes of 2-dodecanol (A), 2-tetradecanol (B), and 2-hexadecanol (C).

nol), 227, 165 ($C_6H_5-CH(CH_3)-NH-CO-OH$), 164 ($C_6H_5-CH(CH_3)-NH-CO-O\cdot$), 147 (165–18; $C_6H_5-CH(CH_3)-N=C=O$), 132 (147–15; $C_6H_5-\dot{C}H-N=C=O$), and 120 ($C_6H_5-CH(CH_3)-NH\cdot$).

Separations of the diastereoisomeric urethanes of 2-dodecanol, 2-tetradecanol and 2-hexadecanol, and of 2-eicosanol and 2-tetracosanol are given in fig. 2 and fig. 3, respectively. In the latter case a small excess of the 2(*R*)-alkanols were added to the racemic 2-alkanols. As shown in fig. 3, also in the case of the 2-alkanols the derivatives of the 2(*S*)-hydroxy compounds had shorter retention times than the derivatives of the corresponding 2(*R*)-hydroxy compounds. The mass spectra of the derivatives of 2-hexadecanol showed ions of high intensity at *m/e* 389 (M), 374 (M-15; loss of $\cdot CH_3$), 330, 240 (M-149;

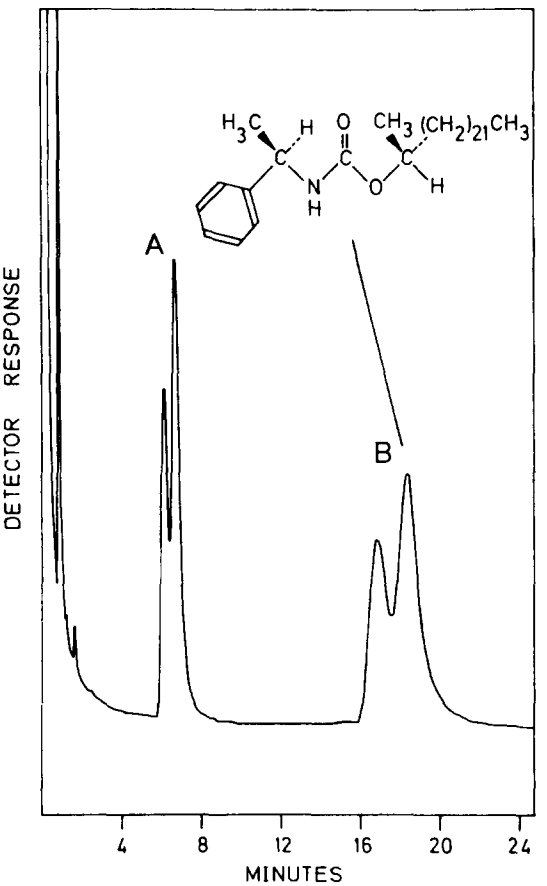

Fig. 3. Separation of the *N*-(1-phenylethyl)urethanes of 2-eicosanol (A), and 2-tetracosanol (B). In both cases a small excess of the 2 (*R*)-alcohols was used. The structure of the derivative of 2 (*R*)-tetracosanol is given.

loss of $C_6H_5-CH(CH_3)-NH-CO\cdot$ plus $H\cdot$), 227, 224 (M-165; loss of $C_6H_5-CH(CH_3)-NH-CO-OH$), 196, 165 ($C_6H_5-CH(CH_3)-NH-CO-OH$), 164 ($C_6H_5-CH(CH_3)-NH-CO-O\cdot$), 150 (165–15; $C_6H_5-\dot{C}H-NH-CO-OH$), 147 (165–18; $C_6H_5-CH(CH_3)-N=C=O$), 132 (147–15; $C_6H_5-\dot{C}H-N=C=O$), and 120 ($C_6H_5-CH(CH_3)-NH\cdot$).

The degree of resolution achieved for the diastereoisomeric urethanes described (separation factor 1.08–1.12, table 1) is of the same order of magnitude as that recently reported for the diastereoisomeric derivatives formed by treatment of a number of hydroxy and amino compounds with (-)-menthyl chloroformate[18]). However, the menthyl formate esters of compounds containing the $-CH(OH)CH_3$ group could not be separated by gas-liquid chromatography using the conditions described above.

Use of the present method has permitted steric analysis of a number of ω2-hydroxy acids produced by enzymatic ω-hydroxylation. The results will be published shortly[19]).

Acknowledgements

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